Pheromones and colonization: reassessment of the milkweed bug migration model (Heteroptera: Lygaeidae: Lygaeinae)*

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Summary. Research on insect migration has justifiably emphasized females – the so-called "oogenesis-flight syndrome" – since it is the females that place the eggs into new habitats. The large and small milkweed bugs, *Oncopeltus fasciatus* and *Lygaeus kalmii*, respectively, have featured prominently in studies of insect migration and sequestration of host plant toxins for chemical defense. Here we report that males of these species, and males of another well-studied lygaeine (*Neacoryphus bicrucis*), produce pheromones in glands usually considered to serve only a defensive role in Heteroptera (the metathoracic scent glands), and that these pheromones are exploited by a tachinid parasitoid as a host-finding kairomone. The pheromones are mixtures of C_6 and C_8 saturated and unsaturated esters reminiscent of lepidopteran pheromones, and the key compound of the *O. fasciatus* pheromone has now been correctly identified as (*E*)-2,7-octadienyl acetate. It is proposed that the concept of the oogenesis-flight syndrome for these kinds of insects should accommodate the role of males in the migration process. The hypothesis is presented that male-produced pheromones play a significant role in guiding colonization of new habitats in many heteropteran species. In addition, data are presented suggesting that there is a trade-off between the amount of pheromone produced by colonizing males and the host breadth of the species.

Key words. insect-plant interactions – parasitoid host-finding – mating strategy – *Oncopeltus*

Introduction

The large milkweed bug, *Oncopeltus fasciatus* (Dallas) (Hemiptera: Heteroptera: Lygaeidae), is a commercially available insect and, despite being of no economic significance, has long been considered a "white rat" in entomology (Feir 1974). This "true bug" is particularly famous in two research arenas: migration and sequestration (Slater & O'Donnell 1995). Milkweed species (Asclepiadaceae) commonly occur in isolated patches, and the bugs must find and colonize these patches to feed on seeds for optimal growth and reproduction (Ralph 1977). Experimentation on the large milkweed bug has highlighted the importance of migration as a life history strategy of young insects to escape adverse conditions and colonize newly available habitats (Dingle 1972, 1996). Analogously, the interaction of *O. fasciatus* with milkweed has been a model system for studying the sequestration of chemicals for defense in insects, in this case cardiac glycosides (Scudder *et al.* 1986; Detzel & Wink 1995). To date, however, these two avenues of *Oncopeltus* research have progressed in parallel, with little or no attempt to relate one to the other.

Another more obscure line of milkweed bug research that nonetheless has a long history of exploration concerns the biosynthesis of exocrine compounds. Like most immature bugs, *Oncopeltus* nymphs have large dorsal abdominal glands that secrete blends of C_6 and C_8 (*E*)-2-alkenals and 4-oxo-(*E*)-2-alkenals for defense (Games & Staddon 1973b). The abdominal glands in young bugs cease to function upon metamorphosis at which time the metathoracic scent gland becomes active (Staddon 1995). This adult-specific gland essentially consists of a pair of lateral tubular glands where esters are commonly produced, and a median reservoir where the more irritating aldehydes are derived by enzymatic hydrolysis of the esters and oxidation of the alcoholic products (Aldrich *et al.* 1978; Staddon & Daroogheh 1981). In most Heteroptera the metathoracic glands look the same in males and females, and the aldehydic secretion from the median reservoir is used solely to combat predators (Aldrich 1988). However, in *O. fasciatus* males the secretion from the tubular glands is somehow withheld from the median reservoir so that the tubular glands themselves swell with secretion (Johansson 1957). The sexual dimorphism observed for the metathoracic glands of *O. fasciatus* is, in fact, expressed chemically: irritated female bugs reportedly released a mixture of C_6 and C_8 (*E*)-2-alkenals and (*E,E*)-2,4-alkadienals (> 99%), whereas males released C_6 and C_8 (*E*)-2-alkenyl and

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(*E,E*)-2,4-alkadienyl acetates (10–30%) in addition to the aldehydes (Games & Staddon 1973a). It appears that acquisition of toxins from host plants surpassed the defensive role of the metathoracic scent gland in milkweed bugs, allowing the gland to secondarily evolve a sexual function (Staddon 1986; Aldrich 1988). Nevertheless, the significance of the esters abundantly produced by males of the large milkweed bug has remained a mystery.

Discovery of a potent aggregation pheromone for a little-known Japanese species of milkweed bug, *Tropidothorax cruciger* (Motschulsky) (Heteroptera: Lygaeidae) (Aldrich *et al.* 1997), prompted us to look for aggregation pheromones of New World lygaeines. We chose to focus our investigation on two well-known North American species that differ in the degree of their host-specificity and are far apart in their propensity to migrate: the large milkweed bug, *O. fasciatus*, and the small milkweed bug, *Lygaeus kalmii* Stål. *Oncopeltus fasciatus* is a specialist on *Asclepias* spp., principally *A. syriaca* in the mid-Atlantic region (Ralph 1977), and is considered a long-distance migrant whose adults must annually re-invade the northern parts of their range because they are unable to survive freezing winters (Dingle 1991). *Lygaeus kalmii* prefers to feed on *Asclepias* spp. but is able to survive on non-milkweed seeds and even insect carrion (Fox & Caldwell 1994), and adults of this species overwinter in north temperate regions (Evans 1987). We also present data for two other *Oncopeltus* specialists of Asclepiadaceae, *O. unifasciatellus* Slater and *O. cingulifer* Stål from tropical Central and South America (Slater 1964), and a specialist of *Senecio* spp. (Asteraceae), *Neacoryphus bicrucis* Say (the whitecrossed seed bug), common from Canada to Brazil (Slater 1964; McLain 1992). All these Lygaeinae are unpalatable due to sequestration of cardiac glycosides from Asclepiadaceae or pyrrolizidine alkaloids from Asteraceae. Individuals of these species are aposematically colored red, orange, or yellow with contrasting areas of black or white (Schuh & Slater 1995) and, at least *O. fasciatus* produces a methoxypyrazine warning odor associated with glycoside-laden secretion (Aldrich *et al.* 1997).

We will argue that male-produced pheromones help guide lygaeines' colonization of host plants, in essence linking migration, reproduction, and sequestration in these insects.

Methods

Chemical identification and preparation of suspected pheromones

Neacoryphus bicrucis adults were collected from an undetermined composite species near the Universidade Federal de Viçosa, Minas Gerais, Brazil, in September, 1995. The metathoracic scent glands were dissected from *N. bicrucis* adults and extracted in CH_2Cl_2 for later analysis in the U.S. (Aldrich *et al.* 1997). For males, the tubular glands and secondary reservoir were dissected and extracted separately from the primary reservoir of the metathoracic scent gland complex. The entire gland complex was extracted for females. Samples were analyzed by gas chromatography (GC) on a DB-5™

column (0.25 µm film, 30-m × 0.25-mm ID) in a Varian 3500 GC with helium as carrier (50 cm/sec linear velocity), a temperature program from 50°C for 2 min to 235°C at 15°/min, with a flame ionization detector (FID). GC-MS was performed using a Hewlett-Packard 5890 GC-mass spectrometer (MS) at 70 eV, with an HP-5™ column (0.11 µm film; 25-m × 0.2 mm ID), programmed from 50°C for 2 min to 250°C at 15°/min. Similar analyses were performed for adults of the commercially available strain of *O. fasciatus* (Carolina Biological Supply Company, Burlington, NC), as well as wild adults of *O. fasciatus* and *L. kalmii* collected at the Agricultural Research Center, Beltsville, Maryland. *Oncopeltus unifasciatellus* individuals were collected and extracted at the Universidade Federal de São Carlos, Brazil, in January, 1997, and a single *O. cingulifer* male was obtained from Homestead, Florida, in August, 1997.

We wanted to verify that *Lygaeus*, *Oncopeltus* and *Neacoryphus* males are smaller than females (as they appear) because smaller body size of male lygaeines translates into faster development from egg to adult (Dingle 1966; Biney 1984). Individuals from new mating pairs from laboratory colonies maintained on milkweed seeds, green beans and water were used for body weight determinations (n = 5/sp.) because this timing ensured that the mass of eggs would be at a minimum, more truly representing body size of females. The quantity of pheromone per male was determined using ethyl (*E*)-4-decenoate as an internal standard (n = 3–5 males/sp.).

Identifications were verified by MS comparisons to, and coinjection with known standards except for 4-oxo-(*E*)-2-octenal which was identified by the published MS (Staddon *et al.* 1985). (*E*)-2,5-Hexadienyl acetate was synthesized by acetylation of (*E*)-2,5-hexadienol with acetic anhydride in pyridine, and purified by flash chromatography on $AgNO_3$-SiO_2 (20% $AgNO_3$). (*E*)-2,5-hexadienol was obtained from lithium aluminum hydride reduction of 5-hexen-2-yn-1-ol which was prepared from propargyl alcohol and allyl bromide according to the method of Jeffrey (1989). (*E*)-2,7-Octadienyl acetate, (*E,E*)-2,4-hexadienyl acetate, phenethyl acetate, and 4-oxo-(*E*)-2-hexenal were synthesized as previously described (Aldrich *et al.* 1997), as were (*E,E*)-2,6- and (*E,Z*)-2,6-octadienyl diacetates and the aldehydes of the corresponding alcohols (Aldrich *et al.* 1996). (*E,E*)-2,4-Octadienyl acetate was prepared from (*E,E*)-2,4-octadienol and acetic anhydride by the same standard procedure. The following standards were purchased: (*E*)-2-hexenyl butyrate, and (*E,E*)-2,4-hexadienol (Aldrich Chemical Company, Milwaukee, WI); (*E*)-2-hexenal, (*E*)-2-octenal, (*E,E*)-2,4-hexadienal, (*E,E*)-2,4-octadienal, (*E,E*)-2,4-octadienol, (*E*)-2-heptenyl acetate, (*E*)-2-octenyl acetate, and ethyl (*E*)-4-decenoate (Bedoukian Research Inc., Danbury, CT). Pheromones were prepared by combining synthetic standards in volumes proportional to each compound's percent abundance as determined by GC (Table 1). A succession of pheromone blends were prepared, based on progressively more detailed chemical analyses and availability of synthetic standards, in an effort to increase species specificity of the pheromones.

Field testing

Field bioassays for the temperate lygaeines were conducted at the Beltsville Agricultural Research Center from June 28 through November 15, 1996, May 2 through November 4, 1997, and March 23 through August 7, 1998. Traps were made of transparent cylindrical containers (20.2 × 19.7 cm; Tri-State Molded Plastics, Inc., Dixon, KY) by cutting two holes (9 cm diameter) in opposite sides, and covering each hole with an inwardly projecting screen funnel (Aldrich *et al.* 1984). In 1996 and 1997, traps were hung ca. 1.5 m from the ground and 15 m apart along a chain-link fence bordering an electrical powerline (alternating treatments; 3/treatment, plus 3 unbaited controls). In 1998, traps were deployed as in previous years except that traps within each set of treatments were hung 5 m apart, and sets were separated by about 30 m to create blocked treatments. The vegetation under the powerline is maintained as a meadow by occasional mowing, and milkweed was scarce to nonexistent along the portion of the fence used for traps. The opposite side of the fence is USDA agricultural land; in 1996 the adjacent field was corn, in 1997 soybeans were planted in this field, and in 1998 corn was planted. Pheromone traps were baited with 5 µl of neat pheromone on a rubber septum (5 × 9 mm; Thomas Scientific, Philadelphia, PA),

monitored every 1–2 days, and rebaited every 2–3 days. Testing dates for the various pheromone blends are indicated in Table 1. Trapped insects were collected alive, and brought to the laboratory for sex determination, rearing, emergence of parasitoids, and dissection. Weather data were recorded every 15 min at a station ca. 200 m from the study site.

A visit by one of us (JRA) to the Universidade Federal de São Carlos provided an opportunity to test lygaeine pheromones in Brazil. After preliminary trials with pheromones for *N. bicrucis*, *L. kalmii*, and *O. fasciatus* it was determined that the *O. fasciatus* blend (Table 1, OF-3) was the most active. Therefore, one trap baited with this blend and one unbaited control trap were deployed in a small patch of weedy vegetation (but lacking milkweed) behind the chemistry building from January 29 through February 8, 1997. Pyramid-shaped baffle traps (Mizell *et al.* 1996) were used except that the collector on top of the trap was a commercially available yellowjacket trap (Sterling International, Inc., Liberty Lake, WA). The collector of the treatment trap was baited daily with a polyethylene pellet saturated with pheromone. Traps were monitored 1–3 times a day, and bugs in or on traps were collected.

Statistical analyses

Analysis of variance was performed on 1997 trap data with the MIXED procedure (SAS Institute Inc. 1997). Residuals were examined graphically for homogeneity and normality. Heterogeneous variance of the residuals was addressed by partitioning the variance into groups of similar variance. Least square means and their standard errors are reported. A Kruskal-Wallis test was used to test whether the numbers of *L. kalmii* and *O. fasciatus* adults caught in pheromone traps in 1997 were identically distributed over the season (CYTEL Software Corp., 1996), and Spearman's rank correlation test was applied to determine if maximum daily temperature was correlated with the number of bugs caught in pheromone traps (Conover 1980).

Results

Chemistry

In early July, 1996, a few individuals of *L. kalmii* were caught in traps baited with the *N. bicrucis* pheromone

(Aldrich *et al.* 1997), and an analysis of these *L. kalmii* males was the basis for the first formulation tested for this species (Table 1). The first blend formulated for *O. fasciatus* was based on an analysis reported earlier in the literature (Games & Staddon 1973a) but it failed to attract any individuals, suggesting essential components were missing. Males from the commercial strain of *O. fasciatus* were obtained and analyzed, revealing that the compound originally identified as (*E,E*)-2,4-octadienyl acetate (Fig. 1) is actually (*E*)-2,7-octadienyl acetate. Once (*E*)-2,7-octadienyl acetate was substituted for (*E,E*)-2,4-octadienyl acetate (Table 1, OF-2), the pheromone was active.

In general, the metathoracic scent gland chemistry of the lygaeines studied reflects their relatedness (Table 2). The tubular glands of males from all five species produce substantial amounts of (*E,E*)-2,4-hexadienyl acetate. Phenethyl acetate is unique to *N. bicrucis*, whereas the *Oncopeltus* and *Lygaeus* tubular gland secretions mainly differ quantitatively. In *L. kalmii*, this secretion is dominated by (*E,E*)-2,4-hexadienyl and (*E*)-2-hexenyl acetates, while in *Oncopeltus* spp. there is much more of the (*E*)-2,7-octadienyl and (*E*)-2-octenyl acetates. (*E*)-2,5-Hexadienyl acetate was present in somewhat higher concentrations in *Oncopeltus* spp. than in *L. kalmii*.

The secretions extracted from the median reservoirs of the lygaeines (Table 2) were the same for males and females (not shown), and devoid of esters with two exceptions. Both *L. kalmii* and *N. bicrucis* reservoir secretions contained appreciable quantities of (*E*)-2-octenyl acetate which, for the latter species, is all the more surprising because this ester was undetectable in the tubular glands. Otherwise, the hypothesis that aldehydes present in the median reservoir are enzymatically derived from the corresponding tubular gland esters is

Table 1 Pheromone blends prepared to mimic the male tubular gland secretions of *Neacoryphus bicrucis*, *Lygaeus kalmii*, and *Oncopeltus fasciatus* (% by volume)

Compound	Species/Blend						
	N. bicrucis	*L. kalmii*		*O. fasciatus*			
	NB	LK-1	LK-2	OF-1	OF-2	OF-3	OF-4
(*E*)-2-hexenyl acetate	98	76.1	79.5	8	7.8	11.5	7.5
(*E,E*)-2,4-hexadienyl acetate	–	6.1	16.8	6	3.1	35.5	30.1
(*E*)-2,5-hexadienyl acetate	–	–	–	–	–	–	1.2
(*E*)-2-hexenyl butyrate	–	–	0.4	–	–	–	–
(*E*)-2-heptenyl acetate	–	–	0.1	–	–	–	0.1
(*E*)-2-octenyl acetate	–	14.1	2.7	28	24.0	15.7	15.6
(*E,E*)-2,4-octadienyl acetate	–	–	–	58	–	–	–
(*E*)-2,7-octadienyl acetate	–	3.7	0.5	–	65.5	37.3	45.5
phenethyl acetate	2	–	–	–	–	–	–
Test period:	6/28–11/15/96	7/2–11/15/96 & 5/2–7/10/97	7/11–11/4/97 & 3/23–8/7/98	7/7–7/25/96	7/26–8/3/96	8/4–11/15/96 & 5/2–11/4/97	7/22–11/4/97 & 3/23–8/7/98
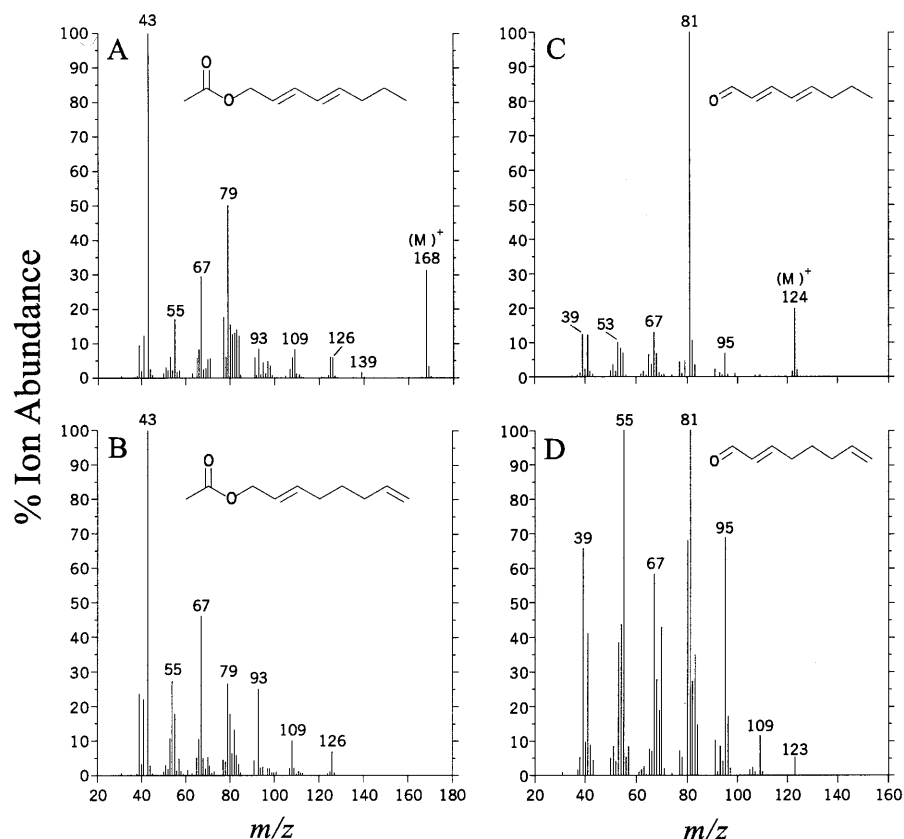
supported by the results shown in Table 2, including the identification of (*E*)-2,7-octadienal (Fig. 1) rather than (*E,E*)-2,4-octadienal as originally reported (Games & Staddon 1973a).

Field data

Neacoryphus bicrucis adults absolutely require phenethyl acetate in their pheromone because no individuals of this species were caught in traps baited with blends mimicking *L. kalmii* or *O. fasciatus* male tubular gland extracts. Captures of *N. bicrucis* males (37) did not significantly exceed that for females (32) ($\chi^2 = 0.36$); however, in the first week in July, 1996, over three times more males than females were captured (Aldrich *et al.* 1997).

For *L. kalmii*, significantly more *L. kalmii* males were caught than females (Table 3). Changing the proportions of components and adding two minor components (LK-2; Table 1) slightly, but significantly increased the specificity of the pheromone blend to *L. kalmii* adults relative to traps baited with the OF-3 blend ($\chi^2 = 14.63$, $P_{0.005} = 7.88$; Figs. 2A and C). However, the increased specificity of the LK-2 versus the OF-3 blend was solely accounted for by increased attraction of males ($\chi^2 = 19.88$); attraction of *L. kalmii* females was not significantly different ($\chi^2 = 0.06$). The highest pheromonal activity for *L. kalmii* was actually to the OF-4 blend which included 1.2% (*E*)-2,5-hexadienyl acetate, and the increased pheromonal activity was

again due to significantly greater attraction of *L. kalmii* males (Table 3). Second- and third-instar nymphs of *L. kalmii* (14) were also caught in pheromone-baited traps from the second week in June through July, 1997.

Fewer *O. fasciatus* adults were attracted to pheromone-baited traps than *L. kalmii* adults, and there were no significant differences in the numbers of male versus female *O. fasciatus* adults caught in pheromone-baited traps (Fig. 2; Table 3). In addition, cross-attraction of *O. fasciatus* to LK-baited pheromone traps was lower than the cross-attraction seen for *L. kalmii* adults. The OF-4 treatment, prepared with the thought that addition of (*E*)-2,5-hexadienyl acetate might increase the species-specificity relative to the OF-3 blend (Table 1), failed to improve attraction of *O. fasciatus* (Table 3).

For *O. unifasciatellus*, the numbers of trapped males (48) and females (40) did not significantly differ ($\chi^2 = 0.73$). One female of *O. varicolor* (F.) was collected on the outside of the pheromone trap in São Carlos.

The parasitic fly, *Leucostoma gravipes* (Wulp) (Diptera: Tachinidae), was caught in traps baited with pheromone blends for *Neacoryphus*, *Lygaeus* and *Oncopeltus* spp. (Table 4). All but one of the trapped *L. gravipes* individuals were female, and female flies were often observed inserting their ovipositor into bugs confined with them in traps. *Leucostoma gravipes* adults were reared from all three lygaeid species. The data for 1996 indicated *L. gravipes* females were more attracted

Table 2 Compounds identified from the tubular glands (T) and median reservoir (R) of the metathoracic scent gland of male *Oncopeltus fasciatus*, *O. unifasciatellus*, *O. cingulifer* (1 male; reservoir not analyzed), *Lygaeus kalmii*, and *Neacoryphus bicrucis* (n = 5 ± SEM, where analyzed individually)

Compound	Species								
	O. fasciatus		*O. unifasc.*		*O. cing.*	*L. kalmii*		*N. bicrucis*	
	T	R	T	R	T	T	R	T	R
(E)-2-hexenyl acetate	7.0 ± 0.2	–	24.0 ± 0.7	–	8.8	79.8 ± 3.7	–	–	–
(E,E)-2,4-hexadienyl acetate	20.3 ± 3.0	–	41.5 ± 2.2	–	38.3	12.5 ± 3.4	–	85.6 ± 3.4	–
(E)-2,5-hexadienyl acetate	1.5 ± 0.4	–	1.9 ± 0.3	–	1.9	0.2 ± 0.04	–	–	–
(E)-2-hexenyl butyrate	–	–	–	–	–	0.3 ± 0.1	–	–	–
(E)-2-heptenyl acetate	0.2 ± 0.1	–	0.2 ± 0.1	–	0.2	0.1 ± 0.04	–	–	–
(E)-2-octenyl acetate	15.3 ± 0.5	–	10.5 ± 1.5	–	7.4	2.6 ± 1.4	9.1 ± 4.6	–	31.8 ± 6.0
(E,E)-2,4-octadienyl acetate	–	–	–	–	–	0.03 ± 0.03	–	–	–
(E)-2,7-octadienyl acetate	45.1 ± 0.1	–	12.3 ± 1.2	–	29.9	0.5 ± 0.3	–	–	–
(E,Z)-2,6-octadienyl diacetate	1.4 ± 0.2	–	1.1 ± 0.3	–	0.2	–	–	–	–
(E,E)-2,6-octadienyl diacetate	0.7 ± 0.4	–	0.8 ± 0.4	–	0.2	–	–	–	–
phenethyl acetate	–	–	–	–	–	–	–	10.1 ± 2.1	–
(E)-2-hexanol	–	–	–	–	–	1.0 ± 0.1	–	–	–
(E)-2-hexenal	–	12.5 ± 1.1	–	24.3 ± 2.4	–	–	23.0 ± 5.0	–	2.2 ± 0.3
(E,E)-2,4-hexadienal	–	4.1 ± 0.3	–	7.6 ± 0.5	–	–	–	–	–
(E)-2-octenal	–	22.3 ± 1.1	–	28.5 ± 1.7	–	–	46.4 ± 6.5	–	9.3 ± 1.4
(E)-2,7-octadienal	–	50.1 ± 1.2	–	26.6 ± 1.2	–	–	–	–	–
(E,Z)-2,6-octadienal	–	1.0 ± 0.1	–	1.1 ± 0.5	–	–	–	–	–
(E,E)-2,6-octadienal	–	3.9 ± 0.5	–	4.2 ± 1.3	–	–	–	–	–
4-oxo-(E)-2-hexenal	–	–	–	–	–	–	9.6 ± 1.9	–	3.1 ± 0.5
4-oxo-(E)-2-octenal	–	–	–	–	–	–	4.0 ± 1.3	–	40.1 ± 4.2
% of Total Volatiles:	91.5	93.9	92.3	92.3	86.9	97.0	92.1	95.7	86.5

to the pheromone formulation for *N. bicrucis* than those of the other two species. In 1997, the OF-4 pheromone significantly increased attraction of the tachinid parasitoid compared to the LK-2 and OF-3 formulations.

The distribution of *L. kalmii* and, to a lesser extent *O. fasciatus*, adults caught in pheromone traps was bimodal in 1997 (Fig. 3). Peak abundances occurred in June and August, with lower trap catches in July. The possibility that hot mid-summer temperatures depressed milkweed bug flight, resulting in the observed bimodal distributions, was considered by analyzing the correlation between trap catches for June through July with temperatures above and below 31°C (a temperature reportedly physiologically damaging to *O. fasciatus*; Dingle 1991). There was a significantly positive correlation between the daily captures of *L. kalmii* adults and temperatures below 31°C (Spearman Correlation Coefficient = 0.26548, $P < 0.05$). However, above 31°C there was no correlation between *L. kalmii* trap catches and maximum daily temperature, and no significant correlations were detected for *O. fasciatus* pheromone-trapping and daily maximum temperatures.

In 1997, traps were deployed on May 2: a female *O. fasciatus* was caught on May 4 and a male was caught on May 9; the first *L. kalmii* adults were caught on

Table 3 Attraction of *Oncopeltus* and *Lygaeus* adults to traps baited with LK-2, OF-3 and OF-4 pheromone formulations, July 22 through November 4, 1997 (treatments as in Table 1)

Effect	Treatment	Gender	Mean/Trap/Day ± SEM	
			O. fasciatus	*L. kalmii*
Treatment	LK-2	–	0.0033 ± 0.0033 a	1.25 ± 0.23 a
Treatment	OF-3	–	0.048 ± 0.011 b	1.17 ± 0.23 a
Treatment	OF-4	–	0.058 ± 0.011 b	2.09 ± 0.23 b
Gender	–	Female	0.031 ± 0.007 a	0.90 ± 0.21 a
Gender	–	Male	0.042 ± 0.007 a	2.11 ± 0.21 b
Trt × Gender	LK-2	Female	NS	0.72 ± 0.26 a
Trt × Gender	OF-3	Female	NS	0.81 ± 0.26 a
Trt × Gender	OF-4	Female	NS	1.16 ± 0.26 a
Trt × Gender	LK-2	Male	NS	1.79 ± 0.26 a
Trt × Gender	OF-3	Male	NS	1.52 ± 0.26 a
Trt × Gender	OF-4	Male	NS	3.01 ± 0.26 b

Numbers in a column followed by a different letter within an effect group are significantly different at the 0.05 confidence level (The Mixed Procedure, SAS Institute Inc. 1997)
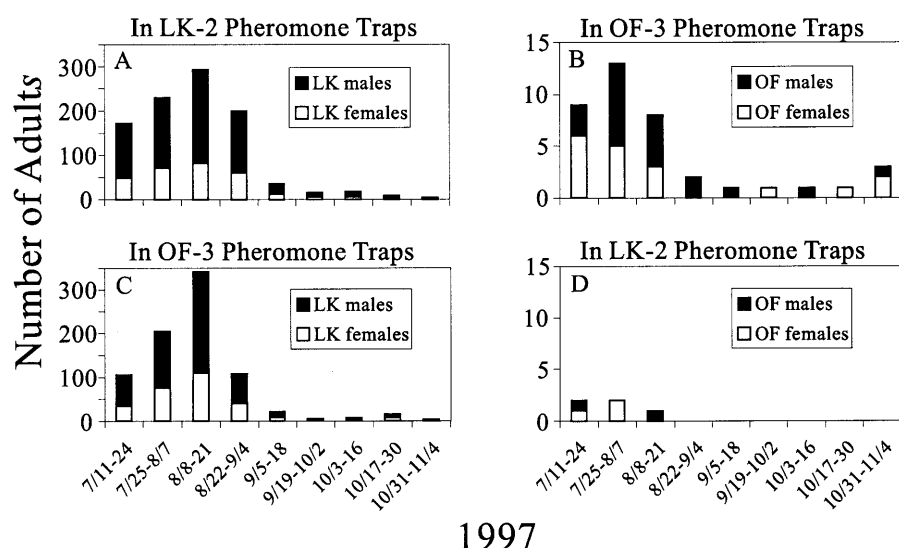
Fig. 2 Adult *Lygaeus kalmii* males and females caught in traps baited with LK (**A**) and OF (**C**) pheromone formulations, and adult *Oncopeltus fasciatus* males and females caught in traps baited with OF (**B**) and LK (**D**) pheromone formulations from July 11 through November 4, 1997 (pheromone formulations as in Table 1)

May 20 (2 males and 3 females). In 1998, traps were deployed on March 23: the first milkweed bugs caught were *L. kalmii* on March 27 (4 males and 3 females); the first *O. fasciatus* individual (a male) was caught on April 15. For 1997, the Kruskal-Wallis test indicated that the distributions of *L. kalmii* versus *O. fasciatus* adults captured in pheromone-baited traps were significantly different (K-W = 8.687, df = 1, P = 0.003), primarily due to relatively higher proportion of *O. fasciatus* individuals caught from mid-June to August 11. In 1998, the distributions of *L. kalmii* versus *O. fasciatus* adults were not significantly different (K-W = 1.533, P = 0.22).

Body weight and pheromone production

Females are larger than males for all of the Lygaeidae studied (Table 5). Pheromone production varied from a maximum of 1.81 μg/mg body weight in *L. kalmii* males to a minimum of 0.21 μg/mg body weight in *O. unifasciatellus*.

Discussion

The "pleasant fruity aroma" from *Oncopeltus fasciatus* (Lener 1967) is, indeed, a pheromone produced in the enlarged tubular glands of males (Johansson 1957). (*E*)-2,7-Octadienyl acetate is the key pheromone component of the large milkweed bug rather than (*E,E*)-2,4-octadienyl acetate as previously reported (Games & Staddon 1973a; Games *et al.* 1974; Everton & Staddon 1979; Staddon *et al.* 1985). The small milkweed bug, *Lygaeus kalmii*, relies on (*E*)-2-hexenyl, (*E,E*)-2,4-hexadienyl, and (*E*)-2,5-hexadienyl acetates for its pheromone, and apparently the tachinid parasitoid, *Leucostoma gravipes*, is also most responsive to these C_6 acetates. The pheromones of lygaeines are analogous to those of many Lepidoptera where particular ratios of unsaturated acetate esters constitute the pheromonal message (Mayer & McLaughlin 1991).

Male lygaeines do not merely search for females. Instead, our data indicate that males often attract females with their pheromone. The fact that males are also caught in pheromone-baited traps very early in the spring, even males of *O. fasciatus* which is considered a long-distance migrant, shows that males themselves are also migrants and suggests that there must be some advantage of a male going to another male's pheromone. The exploitation of these pheromones by parasitoids searching for hosts may have provided the selective pressure favoring a "silent" male counter strategy whereby a male responds to the pheromone of a conspecific in order to intercept incoming females without risking parasitism (Aldrich 1995). The observation that milkweed bug nymphs are attracted to the pheromone of conspecific males provides an additional clue as to how the pheromone system of these insects has evolved: pheromone is associated with the presence of food. We propose that the male-produced pheromones of migratory Heteroptera are an integral part of a resource-based mating system (Thornhill & Alcock 1983) in which a male colonizes a new host (or prey) patch and guides potential mates to the patch with his pheromone.

Appreciation of the role of pheromones in the colonization of new habitats helps explain the behavior of male heteropteran migrants. Males of *O. fasciatus*, as do females, exhibit a peak in flight 8–10 days after

Table 4 Females of the parasitic tachinid fly, *Leucostoma gravipes*, caught in pheromone-baited traps in 1996 and 1997 (treatments as in Table 1; C = control)

Year	Σ *L. gravipes*/Treatment						
	C	NB	LK-1	OF-2	LK-2	OF-3	OF-4
1996	0 a	45 c	11 b	6 ab	–	–	–
1997	0 a	–	–	–	7 b	9 b	18 c

Numbers within a row followed by different letters are significantly different at the 0.05 confidence level (The Mixed Procedure, SAS Institute Inc. 1997)
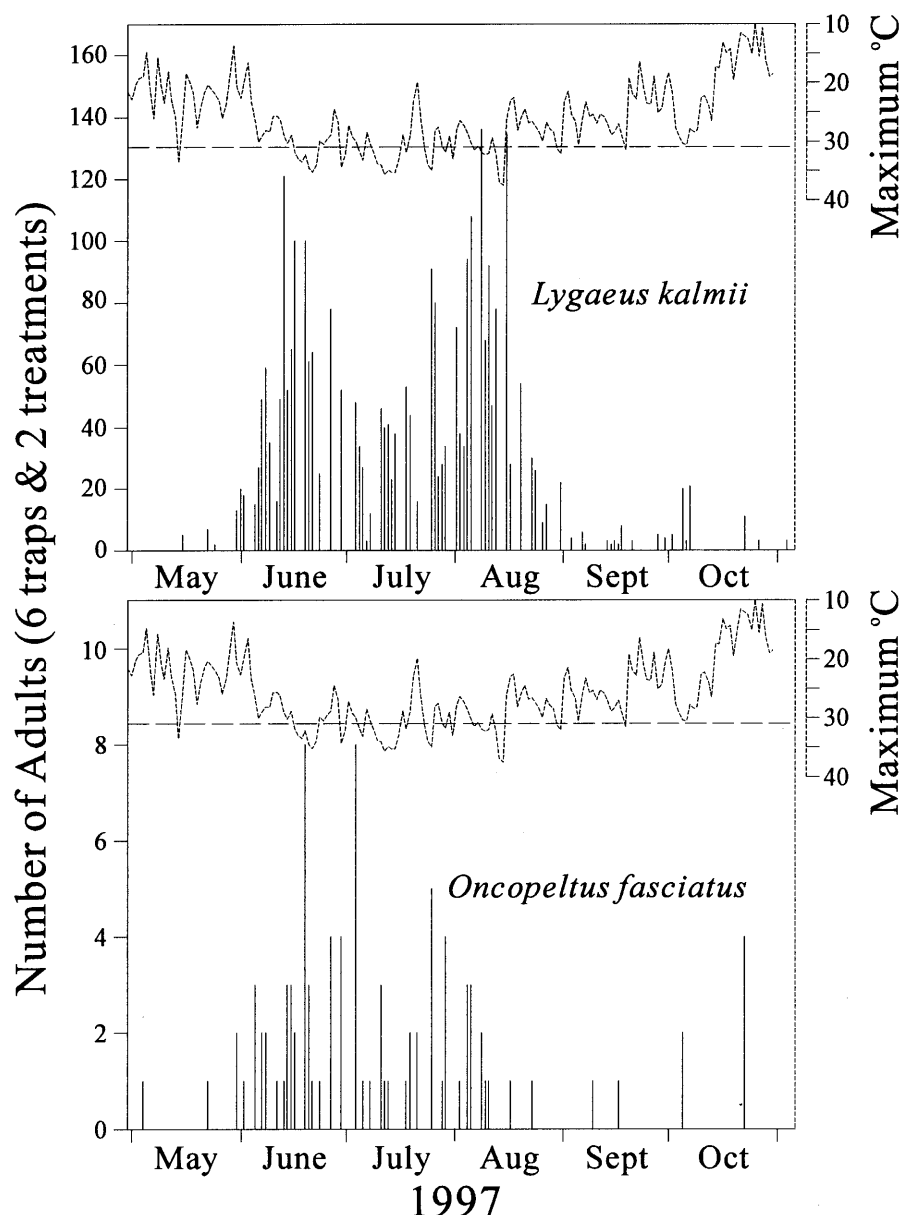
emergence, but older males fly more than females the same age (Dingle 1965) and males live longer than females reared under the same conditions (Dingle 1966). In cotton stainer bugs, *Dysdercus* spp. (Pyrrhocoridae), males evidently produce pheromones (Farine *et al.* 1992) and they are always capable of flight, live longer than females, and migrate to new hosts late in life (Dingle & Arora 1973; Fuseini & Kumar 1975). *Neacoryphus bicrucis* males occupy and defend choice host plants (McLain 1984), probably as they are pheromone-calling. Similarly, *L. kalmii* males persist in milkweed patches as females move through these patches mating and ovipositing (Evans 1987). Continuous short-range movements of the insects likely accumulate to produce long-range displacement of individuals (Evans 1987), and this situation may equally apply to the seasonal range expansions of species such as *O. fasciatus* and *N. bicrucis*.

The smaller body size of male lygaeines translates into somewhat faster development from egg to adult (Dingle 1966; Biney 1984). Therefore, the first individuals from a given clutch of eggs will be mostly males. Upon emergence, if food resources remain abundant, the tendency of the adult bugs to emigrate is suppressed (Dingle & Winchell 1997) but, if food is scarce, a male will search for fresh habitat. For generalist species like *L. kalmii*, the quest for new food plants is, by definition, less restricted than for the more specialized *Oncopeltus* species. As for most phytophagous insects, plant volatiles are undoubtedly important cues to colonizers. Generalists are more apt to be scattered in the environment compared to specialists such as *Oncopeltus* which orient to a narrow range of host plants (Ralph 1976). Consequently, males of generalist species have evolved under a selection regime favoring greater reliance on pheromone which may account for the rela-

Species/Sex	Weight (mg ± SEM)	Pheromone/ Male (µg) (N)	µg Pheromone/ mg Body Weight
L. kalmii/FEMALE	56.4 ± 4.4	–	
L. kalmii/MALE	34.0 ± 0.9	61.44 (5)	1.81
O. fasciatus/FEMALE	62.1 ± 2.7	–	
O. fasciatus/MALE	36.2 ± 2.0	18.53 (5)	0.51
O. unifasc./FEMALE	48.5 ± 3.7	–	
O. unifasc./MALE	29.0 ± 3.3	6.02 (5)	0.21
N. bicrucis/FEMALE	20.4 ± 0.6	–	
N. bicrucis/MALE	10.1 ± 0.3	6.10 (3)	0.60

Table 5 Body weight and pheromone production of *Lygaeus kalmii*, *Oncopeltus fasciatus*, *O. unifasciatellus*, and *N. bicrucis*

tively greater pheromone investment of *L. kalmii* males compared to *Oncopeltus* and *Neacoryphus* males. Predaceous Heteroptera like the spined soldier bug (Pentatomidae: *Podisus maculiventris*) feed on such a wide variety of prey that particular plant volatiles are unreliable indicators of the presence of potential mates, and *P. maculiventris* males produce at least an order of magnitude more pheromone per unit body mass than do lygaeines (Aldrich 1995). In other words, there may be a trade-off between the amount of pheromone needed and the host breadth of the colonizing species.

The older view that insect migration was a desperate attempt to relieve population pressure gave way to a more modern interpretation that insect migrants are colonizers, not merely refugees (Rankin *et al.* 1986; Dingle 1996). Research on insect migration then justifiably focused on females – the so-called "oogenesis-flight syndrome" – since females deposit eggs into new habitats (Johnson 1966). However, now that the male-produced pheromones of milkweed bugs have been characterized and demonstrated to be highly active in the field, the concept of the oogenesis-flight syndrome for these kinds of insects should be expanded to encompass the role of males in the migration/colonization process. The extent to which host-plant physiology and semiochemistry influence pheromone communication, as they do in lepidopterans (*e.g.* McNeil & Delisle 1989; Raina *et al.* 1992), is an important question for future research – after all, most agricultural pests in the Heteroptera migrate into the crops they infest.

Acknowledgements

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